

Applicants: Romero et al.
Serial No.: 10/511,384
Filed: October 15, 2004
Response to Office Action Dated March 12, 2008
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IN THE SPECIFICATION:

Please insert the attached substitute sequence listing after the specification but before the claims.

On pages 18 and 19 of the specification, please replace Tables 1 and 2 with the following new tables:

Table 1. Estimation of the VEGF protein family MHCI associated peptides in the context of HLAA.0201

A.- Using BiMAS software										B.- Using SYPEITHI software										
VEGF-A			VEGF-B			VEGF-C			VEGF-D			VEGF-A			VEGF-B			VEGF-C		
SEQ ID	Secuencia	Kd	SEQ ID	Secuencia	Kd	SEQ ID	Secuencia	Kd	SEQ ID	Secuencia	Kd	SEQ ID	Secuencia	Kd	SEQ ID	Secuencia	Score	SEQ ID	Secuencia	Score
27	LLSWVHWSL	272	37	LLLAALLQL	309	47	YLSKTLFEI	640	57	FMMLYVQLV	1966	67	RLFPCFLQL	150						
28	ALLYLHHA	42	38	Q LAPAQAPV	70	48	T LFEITVPL	324	58	KLWRCRRL	620	68	VSEYPSEV	42						
29	WSLALLYL	30	39	QLVPSCVT	70	49	V LYPEYWKM	304	59	QLFEISVPL	324	69	VMRLFPCFL	42						
30	FLQHNKCEC	23	40	LMGTVAKQL	26	50	C MNTSTS YL	85	60	YISKQLEI	88	70	RALERLVVDV	34						
31	WVHWSSL ALL	20	41	L LAALLQLA	19	51	KLFPSQCGA	64	61	C MNTSTS YI	41	71	VELTFSQHV	32						
32	FLLSWVHWS	16	42	L LQ LAPAQ A	8	52	L LGFFSVAC	32	62	V LQEENPLA	35	72	AVPPQQWAL	14						
33	RQLELNERT	6	43	V VSWIDVYT	6	53	S LPATLPQC	11	63	WVVVVNVFM	27	73	L QLLAGLAL	14						
34	NITMQIMRI	3	44	C VPTGQHQV	6	54	G LQCMNTST	7	64	V NVFMMLYV	10	74	RSGDRPSYV	10						
35	YCHPIETLV	2	45	K QL VPSCVT	4	55	A AFESGIDL	4	65	S LICMNTST	7	75	LLAGLALPA	8						
36	I EYIFKPSC	2	46	V VVPLTVEL	3	56	E QLRSVSSV	4	66	C VLQEENPL	7	76	CPV VETAN V	6						

Note: Values in bold correspond to those peptides or their regions, which coincide in both predictions.

Table 2. Estimation of VEGF family receptors MHCI associated peptides in the context of HLA.A0201

A.- Using BiMAS software										NRP-2		
VEGFR-1			VEGFR-2			VEGFR-3			NRP-1		NRP-2	
SEQ ID	Secuencia	Kd	SEQ ID	Secuencia	Kd	SEQ ID	Secuencia	Kd	SEQ ID	Secuencia	Kd	SEQ ID
127	FLYRDTWV	1942	137	VLLWEIFSL	1792	147	VLLWEIFSL	1793	157	GLLRFVTAV	2249	167
128	VLLWEIFSL	1792	138	SLQDQGDYY	769	148	RLLEEKSGV	1055	158	VLLGAVCGV	1006	168
129	KLLRGHTLV	901	139	VLLAVALWL	739	149	VLWPDGQEV	981	159	WMPENIRLV	436	169
130	GLLTCEATV	257	140	AMFFWLLLV	427	150	NLTDLLYNV	656	160	GILSMVFTT	278	170
131	TLFWLLLTL	182	141	VIAIFFWLL	270	151	KQAERGKWW	557	161	LLCAVLALV	272	171
132	ILSENNVV	179	142	ILLSEKNNV	179	152	GVIAVFFWW	369	162	VLLHKSLKL	134	172
133	TLNLTIMNV	160	143	LLAVALWLC	146	153	KLVQIANNV	243	163	GMLGMVSGL	131	173
134	CVAATLFWL	137	144	KNLDTLWKL	128	154	ALWNSAAGL	177	164	FQLTGTTTV	120	174
135	LLSIKQSNV	118	145	AVIAMFFFWL	113	155	TLSLSPRV	160	165	VLAKEKPTV	118	175
136	SLQDSGTYA	112	146	LLLVIILRT	108	156	SQHDLGSYV	159	166	GPFLFIKFV	81	176
B.- Using SYFPEITHI software										NRP-2		
VEGFR-1			VEGFR-2			VEGFR-3			NRP-1		NRP-2	
SEQ ID	Secuencia	Score	SEQ ID	Secuencia	Score	SEQ ID	Secuencia	Score	SEQ ID	Secuencia	Score	SEQ ID
177	TLFWLLLTL	29	187	VLLWEIFSL	29	197	VLLWEIFSL	29	207	VLLGAVCGV	30	217
178	VLLWEIFSL	29	188	LLVILRLTV	28	198	SIPGLNVTL	27	208	GLLRFVTAV	29	218
179	ILGPGSSTL	28	189	GLFCKTLTI	26	199	NLTDLLYNV	27	209	LLCAVLALV	28	219
180	LLCALLSCL	27	190	SIMYVWWV	26	200	VLWPDGQEV	26	210	GMLGMVSGL	28	220
181	GLLTCEATV	27	191	ILVGTAI	26	201	LLPRKSLEI	26	211	ALGVLLGAV	28	221
182	LLRGHTLV	27	192	ALMSELKIL	26	202	ALWNSAAGL	26	212	VLLHKSLKL	27	222
183	ALMTTELKIL	26	193	AASVGLPSV	25	203	IMDPGEVPL	26	213	VLAKEKPTV	26	223
184	KLLRGHTLV	25	194	SISNLNVSL	25	204	RLWLCLGLL	25	214	QLTGTTTV	25	224
185	TLNLTIMNV	25	195	AMFFWLLLV	25	205	LIVFYVTTI	25	215	VLLGAVCGV	30	225
186	ILSENNVV	25	196	ILLSEKNNV	25	206	LLEGQPVLL	25	216	GLLRFVTAV	29	226
												GIGMRLEV

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On page 21 of the specification, please replace the paragraph beginning on line 9 with the following:

In the case of the extracellular domains 1 to 3 SEQ ID NO: ~~27~~ ~~23~~ and SEQ ID NO: ~~28~~ ~~24~~ (~~for domains 1-3~~) and ~~SEQ ID NO: 29 and SEQ ID NO: 30 (for domain 3 alone)~~, the primers used correspond to sequences SEQ ID NO: 9 and SEQ ID NO: 10. After digestion of the amplified fragment (943bp) ~~SEQ ID NO: 25 and SEQ ID NO: 26~~ with endonucleases BamHI and EcoRI, the cDNA coding 1-3 domains of KDR was purified, and cloned in pAECΔ2 vector. Clones positive by restriction analysis were verified by sequencing of the corresponding DNA. The cDNA corresponding to KDR 1-3 was then subcloned KpnI/EcoRV in the already described pMAE5Δ5 vector (pMAE5Δ5 KDR1-3).

For the cloning of transmembrane and cytosolic regions of the receptor (SEQ ID NO: 25 and SEQ ID NO: 26) a two-step strategy was designed. For the insertion of the first segment, the primers corresponding to SEQ ID NO: 11 and SEQ ID NO: 12 were used. After the XbaI/BglIII digestion of this 747bp segment, the product was cloned in the pMAE5 vector, previously digested with the same enzymes, obtaining the plasmid PMAE5 KDR 747. This plasmid was digested BglIII/NotI in order to insert the remaining carboxy-terminal fragment of 1091bp that was amplified using the primers corresponding to sequences SEQ ID NO: 13 and SEQ ID NO: 14. Clones positive by restriction analysis were verified by DNA sequencing and denominated pMAE5 KDR C.